Trans-Suppression of Terminal Deficiency-Associated Position Effect Variegation in a Drosophila Minichromosome

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ABSTRACT

Position effect variegation (PEV) is the clonal inactivation of euchromatic or heterochromatic genes that are abnormally positioned within a chromosome. PEV can be influenced by modifiers in *trans*, including single gene mutations and the total amount of heterochromatin present in the genome. Terminal deletions of a Drosophila minichromosome (*Dp1187*) dramatically increase PEV of a *yellow*$^+$ body-color gene located in *cis*, even when the terminal break is >100 kb distal to the *yellow* gene. Here we demonstrate that terminal deficiency-associated PEV can be suppressed by the presence of a second minichromosome, a novel phenomenon termed "*trans*-suppression." The chromosomal elements responsible for *trans*-suppression were investigated using a series of minichromosomes with molecularly characterized deletions and inversions. The data suggest that *trans*-suppression does not involve communication between transcriptional regulatory elements on the homologues, a type of transvection known to act at the *yellow* locus. Furthermore, *trans*-suppression is not accomplished by titration through the addition of extra centric heterochromatin, a general mechanism for PEV suppression. We demonstrate that *trans*-suppression is disrupted by significant changes in the structure of the suppressing minichromosome, including deletions of the *yellow* region and centric heterochromatin, and large inversions of the centric heterochromatin. We conclude that chromosome pairing plays an important role in *trans*-suppression and discuss the possibility that terminal deficiency-associated PEV and *trans*-suppression reflect changes in nuclear positioning of the chromosomes and the gene, and/or the activity and distribution of telomere-binding proteins.

METAZOAN chromosomes are composed of heterochromatin and euchromatin. Heterochromatin is highly condensed throughout the cell cycle, including interphase, is composed primarily of highly and middle-repetitive DNA sequences, and contains relatively few mutable genes (GATTI and PIMPINELLI 1992; LOHE and HILLIKER 1995). Euchromatin is decondensed during interphase and contains most of the single-copy DNA in the genome. Juxtaposition of euchromatin and heterochromatin can induce clonal inactivation of both heterochromatic and euchromatic genes, a phenomenon known as position effect variegation (PEV) (for review, see LEWIS 1950; HENIKOFF 1990; KARPEN 1994; WEILER and WAKIMOTO 1996). Heterochromatin-induced PEV of euchromatic genes is suppressed by the addition of an extra *Y* chromosome and is modified by a large number of loci that encode suppressors and enhancers of variegation (Reviewed by GRIGLIATTI 1991). Studies of PEV suggest that long-range chromosomal effects influence the expression of individual genes (for review, see KARPEN 1994; WEILER and WAKIMOTO 1996). Such effects could be acting either in *cis* (along a chromosome) or in *trans* (between homologous chromosomes).

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Cytogenetic analyses carried out over the past 70 years have led to the suggestion that PEV of euchromatic genes could be caused by "heterochromatic spreading" in *cis*. Formation of heterochromatin structure may begin within heterochromatic DNA and spread until a termination sequence is encountered or until heterochromatic factors have been depleted. A euchromatic gene inappropriately positioned near heterochromatin would then be inactivated by inclusion in the spreading heterochromatin, on a cell-by-cell basis. Although heterochromatic spreading is consistent with changes in gross chromosome structure (SCHULTZ 1936; PROKOFYEVA-BELGOVSKAYA 1939) and the distance-dependence of inactivation observed in variegating rearrangements (DEMEREC 1941), there is no direct proof that heterochromatic spreading causes PEV (reviewed in SPRADLING and KARPEN 1990; KARPEN 1994; HENIKOFF 1996).

In recent years alternative models have been proposed to explain PEV as a long-range *cis*-acting phenomenon. In some Drosophila variegating rearrangements, heterochromatic and nearby euchromatic DNA is underrepresented in polytene tissues (KORNHER and KAUFFMAN 1986; KARPEN and SPRADLING 1990), possibly due to somatic elimination or underreplication (SPRADLING and KARPEN 1990). In these cases, variega-

tion phenotypes likely result from reduced gene dosage. However, investigation of other variegating alleles has shown no evidence for underrepresentation (HAYASHI *et al.* 1990; ZHANG and SPRADLING 1995; WALLRATH *et al.* 1996), indicating that underrepresentation is not the only mechanism for PEV.

Nuclear organization and gene localization within nuclear domains may also play an important role in PEV and the long-range regulation of gene expression. Cytological analyses reveal that nuclei frequently exhibit a reproducible organization during interphase (for review, see HAAF and SCHMID 1991; DERNBURG *et al.* 1995). One example of nuclear organization is the Rabl configuration (RABL 1885) displayed by some interphase nuclei, including those of the Drosophila embryonic and salivary gland tissue (HOCHSTRASSER *et al.* 1986). In these cells, telomeres and centromeres (which are constitutively heterochromatic) are clustered at opposite sides of the nucleus while the euchromatic portion of the genome is located predominantly in the nuclear lumen (MATHOG *et al.* 1984; HOCHSTRASSER *et al.* 1986; FUNABIKI *et al.* 1993). Each chromosome inhabits a unique domain within the nucleus (MATHOG *et al.* 1984; HOCHSTRASSER *et al.* 1986). In addition, some specific euchromatic loci, as well as regions that may function as interstitial heterochromatin, appear to be associated with the nuclear membrane (HOCHSTRASSER *et al.* 1986; MARSHALL *et al.* 1996) while other loci are consistently located far from the nuclear membrane (MARSHALL *et al.* 1996). Although not all nuclei exhibit a Rabl configuration, most seem to be organized in a reproducible and specific fashion: each chromosome still inhabits a specific, unique domain within the nucleus (LICHTER *et al.* 1988; MANUELIDIS and BORDEN 1988; reviewed in HAAF and SCHMID 1991). In Drosophila diploid larval neuroblasts and imaginal discs, heterochromatin seems to be associated in multiple, distinct regions (DERNBURG *et al.* 1996) and telomeres are associated with each other in *Saccharomyces cerevisiae* nuclei (KLEIN *et al.* 1992).

Genetic studies provide evidence that the organization of chromosomes and the position of genes in nuclei may impact gene expression. In Drosophila, rearrangements that move genes ordinarily found in heterochromatin (such as *light* and *rolled*) away from their normal chromosome locations cause clonal suppression of their function (SCHULTZ and DOBZHANSKY 1934; EBERL *et al.* 1993). Heterochromatic gene PEV can be relieved by secondary rearrangements that move the variegating gene more proximal on the chromosome arm, suggesting that heterochromatic genes require a specific nuclear position to be appropriately regulated (WAKIMOTO and HEARN 1990; EBERL *et al.* 1993). Conversely, variegation of a euchromatic gene, *brown* (*bw*), is enhanced by rearrangements that move the variegating locus to a more proximal position within the euchromatin and is suppressed by aberrations that move it to a more distal position (TALBERT *et al.* 1994). These observations, and the cytological studies described above, have led to a model that specific heterochromatic and euchromatic domains exist within the nucleus, and that positioning a gene within an inappropriate domain can inhibit gene function (WAKIMOTO and HEARN 1990; EBERL *et al.* 1993; KARPEN 1994; HENIKOFF *et al.* 1995). Functional nuclear domains could be determined by interactions between specific chromosomal regions or by absolute nuclear position within the three-dimensional nucleus.

Recent cytological studies of PEV at the *brown* (*bw*) locus provide support for the general idea that altering the position of a gene within the nucleus, relative to other chromosomal regions, affects its expression. bw^D PEV results from insertion of a large piece of heterochromatin within the *bw* coding region. The heterochromatin disrupts *bw* expression in *cis* and also acts dominantly in *trans* to induce PEV of the homologous bw^+ gene (HENIKOFF and DREESEN 1989). *In situ* hybridization to whole nuclei with probes from the *bw* region and heterochromatic satellite DNAs indicate that in bw^D, the transposed heterochromatin and the adjacent *bw* locus are frequently located in close proximity to centric heterochromatin on the same autosome (CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996). The association of the *bw* locus with centric heterochromatin is sensitive to modifiers of PEV (CSINK and HENIKOFF 1996). Such data are consistent with a model that bw^D PEV occurs because the bw^+ allele is inappropriately positioned within one of the heterochromatic domains of the nucleus.

Gene regulation can also be affected by pairing of homologous chromosomes (PIRROTTA 1990; TARTOF and HENIKOFF 1991; WU 1993). For example, transvection occurs when the enhancer of one gene activates the promoter of the other allele present on the homologous chromosome. Transvection acts at a number of loci, including *Ultrabithorax* (LEWIS 1954; CASTELLI-GAIR *et al.* 1990; MICOL *et al.* 1990; MARTINEZ-LABORDA *et al.* 1992), *decapentaplegic* (GELBART 1982), *white* (GUBB *et al.* 1990), and *yellow* (GEYER *et al.* 1990). Rearrangements that alter chromosome pairing disrupt transvection. Chromosome pairing can also play a role in PEV. The cytological studies described above (CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996) indicate that the bw^D allele can pair with the bw^+ locus on the homologous chromosome, causing the bw^+ locus to also be associated with the centric heterochromatin. bw^D PEV can be prevented by chromosomal changes that interfere with the ability of the two chromosomes to pair, suggesting somatic pairing plays an important role in the phenomenon (HENIKOFF and DREESEN 1989). Chromosome pairing also affects the PEV of *P*-element marker genes inserted into subtelomeric regions. Such variegation can be suppressed by heterozygosity with a chromosome that has a terminal deficiency of the re-

gion homologous to the insertion site (LAURENTI *et al.* 1995).

Here we describe and characterize a novel example of long-distance gene regulation that operates both in *cis* and in *trans*. This system has been identified through studies of the Drosophila minichromosome *Dp(1;f)1187* (referred to as *Dp1187*). Previous studies demonstrated that minichromosomes with single breaks that remove the chromosome end (terminal deficiencies) variegate for the *yellow* body color gene, even when the breakpoint is located >100 kb distal to the affected gene (TOWER *et al.* 1993; ZHANG and SPRADLING 1993). Chromosome ends are important for chromosome integrity and function (for review, see ZAKIAN 1995). Loss of terminal structures may affect gene expression by altering the nuclear position of nearby genes or by changing telomere-associated chromatin structure. We report here that the presence of a second minichromosome significantly suppresses terminal deficiency-associated *yellow* PEV (termed "*trans*-suppression"). Molecular-genetic dissection of the chromosomal elements that mediate *trans*-suppression demonstrate that *trans*-suppression does not involve cross-homologue communication between transcription regulatory elements, a type of transvection known to act at the *yellow* locus. Nor is *trans*-suppression accomplished by titration of heterochromatic factors through the addition of extra centric heterochromatin, a general mechanism for PEV suppression. Our data indicate that full *trans*-suppression requires structural homology between the two minichromosomes, suggesting that pairing of the chromosomes is required for full *trans*-suppression. We discuss the possibility that *trans*-suppression reveals how chromosome and gene function are regulated by nuclear positioning and/or the packaging of chromosome ends by telomeric proteins.

MATERIALS AND METHODS

Drosophila stocks and cultures: Flies were grown on standard corn meal/agar medium. *Dp 8-23, Dp 8-23* γ-derivatives and *γ238*-derivatives are described in KARPEN and SPRADLING (1990), TOWER *et al.* (1993), LE *et al.* (1995), and MURPHY and KARPEN (1995). Minichromosomes were maintained as monosomes in a $y^1;ry^{506}$ background. Crosses were done at 22°. All other mutations are described in LINDSLEY and ZIMM (1992).

Measuring *trans*-suppression: To quantitate the level of *yellow*$^+$ (y^+) expression, single virgin females carrying one copy of the *γ878* minichromosome (y± ry− phenotype) were crossed to single males containing a different *Dp1187* derivative (y− ry+ phenotype). To avoid potential problems that multiple derivatives (*e.g.*, *γ878/γ878/γ158*) could create, progeny were scored to determine the transmission frequency of each derivative from the monosomic parents. Only crosses that showed transmission frequencies expected for parents containing a single derivative were selected for further analysis (*e.g.*, 50% for *γ158*, 25% for *26C*; see MURPHY and KARPEN 1995). Progeny were stored in 100% isopropanol at room temperature until the wings were removed and mounted in Gary's Magic Mountant [1.5 g/ml Canada Balsam in methyl salicylate (LAWRENCE 1986)]. Slides were pressed overnight at 45°. y^+ expression was quantitated by counting the number of y+ (black) triple row bristles (see KARPEN and SPRADLING 1990) as a fraction of the total. For each cross, animals with two minichromosomes (y+ ry+) were analyzed to test for the amount of *trans*-suppression, and *γ878* monosomic siblings (y± ry−) were analyzed to control for environmental conditions. At least 50 wings (>4000 triple row bristles) from each class were scored in each experiment. *Trans*-suppression data are presented as values relative to the amount of *trans*-suppression provided by *γ158*. For example, derivatives that suppress equally as well as *γ158* are defined as providing 100% relative suppression.

Previous data indicated *Dp1187* y^+ expression to be 95% wild type (KARPEN and SPRADLING 1990), although in the current study *Dp1187* y^+ expression has been found to be 75% wild type. This difference is likely due to environmental differences or the accumulation of genetic modifiers.

Southern analysis of *yellow* derivatives: The structures of minichromosomal *y* region DNA were determined in a *y ac;*ry^{506} background; ~20 kb of the endogenous *X*-linked *y* gene region is deleted in *y ac* (CAMPUZANO *et al.* 1985). One to 2 μg of genomic DNA [prepared from adults (BENDER *et al.* 1983)] were digested at 37° overnight and electrophoresed through a 0.7–1.0% LE agarose gel, with 1 μg/ml EtBr in both the gel and the 0.5× TBE running buffer. Blotting and hybridization were performed as described in LE *et al.* (1995). Probe DNA was a 7.4-kb *SalI-BglII* fragment that includes the entire y^+ locus (kindly provided by Dr. PAMELA GEYER).

RESULTS

Terminal deficiency *γ878* shows dramatic position effect variegation: The *yellow*$^+$ (y^+) gene is required for dark pigmentation of the adult body cuticle, wing blades, thoracic and wing bristles, and other ectodermal structures (LINDSLEY and ZIMM 1992). A sensitive measure of y^+ expression in individual cells is provided by the triple row bristles, located on the anterior margin of the wing (Figure 1; also see MATERIALS AND METHODS). Minichromosome *Dp(1;f)1187* (referred to as *Dp1187*) is derived from the full-length *In(1)*sc^8 chromosome (KRIVSHENKO and COOPER 1953, cited in LINDSLEY and ZIMM 1992). *Dp 8-23* was created by the insertion of two *PZ* ry^+ *P*-elements into the euchromatic and subtelomeric regions of *Dp1187* (KARPEN and SPRADLING 1992; TOWER *et al.* 1993). In a *y;ry;Dp* animal the only functional y^+ gene is located on the minichromosome, providing a convenient assay for minichromosome-associated PEV. Juxtaposition of centric heterochromatin 20 kb from the y^+ gene in *In(1)*sc^8, *Dp1187,* and *Dp 8-23* causes slight variegation of y^+ expression (KARPEN and SPRADLING 1990 and references therein). In animals that are monosomic for *Dp 8-23* 75% of the triple row bristles are y+, and most of the abdominal cuticle is also pigmented (Figure 1).

Terminal deficiency derivatives of *Dp1187* show increased variegation relative to the parental minichromosome (TOWER *et al.* 1993; ZHANG and SPRADLING 1993). γ-irradiation of *Dp 8-23* produced *γ878,* a terminal deficiency with a breakpoint 55 kb away from the y^+ locus, and no other detectable structural changes

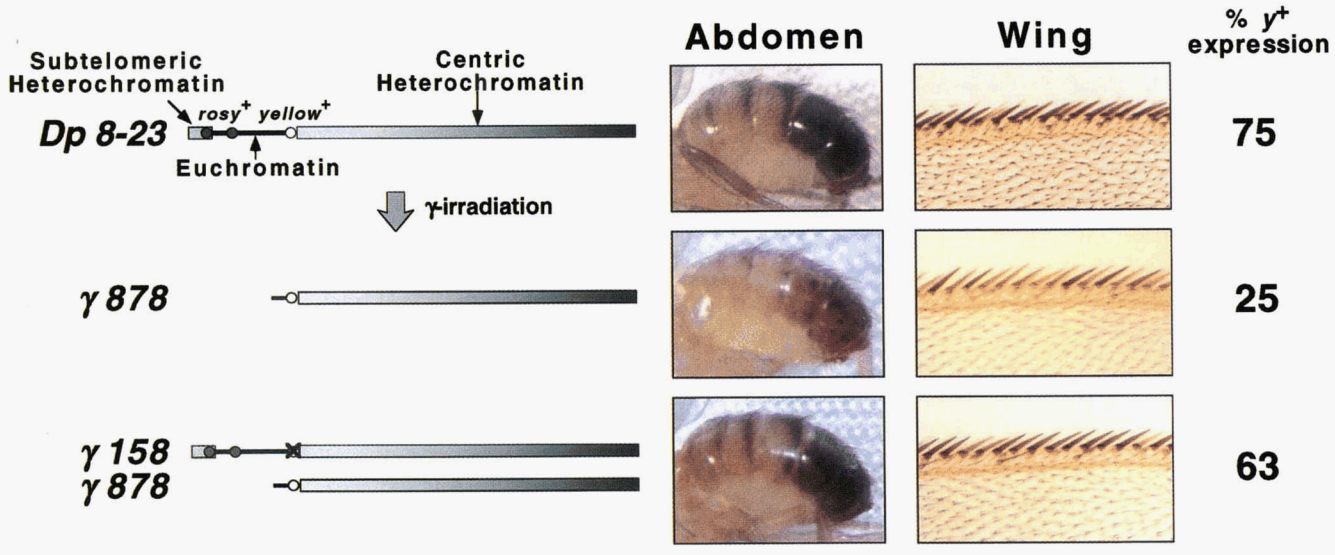

FIGURE 1.—Terminal deficiency-associated *yellow* PEV and *trans*-suppression phenotypes associated with *Dp1187* minichromosome derivatives. *Dp 8-23* is a *P* element insertion derivative of *Dp1187* (KARPEN and SPRADLING 1992; TOWER *et al.* 1993). *γ878* and *γ158* are γ-irradiation derivatives of *Dp 8-23* (LE *et al.* 1995). Structures include centric heterochromatin (shaded box), euchromatin (——), *yellow*$^+$ locus (○), *rosy*+ *P* elements (◎), and subtelomeric heterochromatin (gray box). *yellow*$^+$ variegation phenotype is shown for adult male abdomens and triple row bristles. Level of *yellow*$^+$ expression was quantitated by counting triple row bristles (see MATERIALS AND METHODS).

(LE *et al.* 1995). The y^+ gene present on *γ878* displays severely reduced y^+ expression; only 25% of triple row bristles are y^+ (Figure 1); a similar reduction of the y+ phenotype was observed in the abdominal cuticle (Figure 1).

***γ878* variegation is suppressed by a second minichromosome in *trans*:** *γ158* is a y− ry+ derivative of *Dp 8-23* with no detectable structural changes (Figure 1). Our studies indicate it contains a small mutation in the y^+ gene (a change involving <100 bp, see below). When virgin *γ878* (y± ry−) females are crossed to males containing *γ158* (y− ry+), progeny containing both minichromosomes (y+ ry+) show a dramatic increase in y^+ expression, relative to their *γ878* (y± ry−) siblings (see MATERIALS AND METHODS). Increased y^+ expression is visible in the abdominal cuticle, wings, and thoracic and triple row bristles (Figure 1). Animals with both *γ158* and *γ878* have 63% y+ triple row bristles (a 2.5-fold increase in y^+ expression relative to siblings with only *γ878*), which is very close to the phenotype displayed by intact minichromosomes (75% for *Dp 8-23*) (Figure 1). We refer to the nearly complete suppression of terminal deficiency-induced PEV by a second minichromosome as "*trans*-suppression."

What biological mechanisms could be responsible for *trans*-suppression? Based on published observations, three possible models can be proposed: titration of limited cellular factors by the addition of heterochromatic mass; transvection at the y^+ locus; and chromosome pairing. To test these models, we investigated the level of *γ878* y^+ variegation when in *trans* to other minichromosomes that contain molecularly defined alterations.

***Trans*-suppression of terminal deficiency-associated variegation is not caused by heterochromatin mass titration:** An increase in the overall amount of heterochromatin in the genome has long been known to be a potent suppressor of variegation in *trans* (GOWEN and GAY 1934). In particular, *Dp1187* y^+ variegation is affected by the level of heterochromatin present in the cell: minichromosome-bearing *X*/0 males show substantially reduced y^+ expression relative to *X*/*Y* males (KARPEN and SPRADLING 1990). Therefore we tested the hypothesis that *γ158* causes *trans*-suppression simply because it contributes an additional 1000 kb of centric heterochromatin.

Experiments testing the *trans*-suppressive abilities of derivatives with alterations in the amount of centric heterochromatin indicate that the amount of centric heterochromatin on a minichromosome does not correlate with its ability to *trans*-suppress. Centric heterochromatin is not absolutely necessary for *trans*-suppression, since derivatives with no centric heterochromatin can suppress as well as or better than derivatives with most or all of the 1000 kb of heterochromatin. For example, derivative *26C* is an acentric terminal deficiency, 285 kb in length, that contains no centric heterochromatin (see Figure 2 and MURPHY and KARPEN 1995). *26C* increases *γ878* y^+ expression to 46% of full *γ158* *trans*-suppression (referred to as "relative suppression"; see MATERIALS AND METHODS). This is a level similar to that of *γ1088*, a derivative that contains 1000 kb of heterochromatin (Figure 2). *Dp1187* centric heterochromatin also is not sufficient to produce high levels of *trans*-suppression. A derivative that contains a sub-

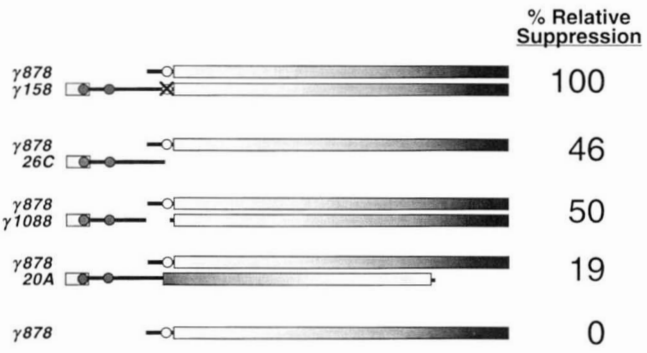

FIGURE 2.—Structures and relative *trans*-suppression of derivatives indicate that centric heterochromatin is neither necessary nor sufficient (see *20A*) for *trans*-suppression. Structures of minichromosomes were determined previously (LE *et al.* 1995; MURPHY and KARPEN 1995). Levels of *trans*-suppression are described relative to that of *γ158* (see MATERIALS AND METHODS). Shading of heterochromatin region indicates orientation.

stantial amount (800 kb) of centric heterochromatin in the inverted orientation (*20A*) only induces 19% relative *trans*-suppression (Figure 2). These results demonstrate that increased mass of heterochromatin is not responsible for *trans*-suppression of *γ878 y*$^+$ variegation.

***Trans*-suppression of variegation is not due to transvection:** Transvection occurs when two independently dysfunctional alleles on homologous chromosomes complement each other to produce a wild-type or nearly wild-type phenotype. Transvection can occur at the *y* locus, between an enhancer on one homologue and the promoter on the other homologue (GEYER *et al.* 1990). This type of transvection only occurs if the promoter in *cis* to the active enhancer is nonfunctional, suggesting that enhancers normally prefer to regulate their own promoter. Chromosomal alterations that separate the two homologues in the region of the gene abolish wild-type expression, suggesting that transvection requires a physical interaction between the two loci.

Careful inspection of the *γ158 y* gene region by Southern hybridization analysis demonstrated that there is no detectable structural change, suggesting that the y− phenotype of *γ158* is caused by a point mutation or small deletion (<100 bp) in its promoter or coding region (Figure 3B). If *γ158* contains a promoter mutation, suppression of *γ878* variegation by *γ158* could be mediated by the type of transvection observed previously at the *y* locus (GEYER *et al.* 1990). Specifically, the *y* enhancers present on *γ158* could be acting to increase *y*$^+$ expression from the *γ878* promoter.

If such a transvection mechanism is responsible for *trans*-suppression, only derivatives with an intact tissue-specific enhancer should be capable of *trans*-suppression in that tissue. Therefore, we tested two y− *Dp 8-23* derivatives with small deletions in the *y* region that eliminated tissue-specific enhancers (GEYER and CORCES 1987) (Figure 3A). The precise nature of the *y* gene mutations in these y− ry+ derivatives were determined by detailed conventional electrophoresis and Southern analyses (Figure 3, B and C, and data not shown). *γ737* has a 1.5-kb deletion that removes part of the first exon and the entire region known to contain the bristle enhancer [which is required for triple row bristle pigmentation; GEYER and CORCES (1987)], and *γ648* has a 2.6-kb deletion of the body enhancer, the promoter, and part of the first exon (Figure 3C).

We examined the ability of *γ737* and *γ648* to *trans*-suppress *γ878* terminal deficiency-associated PEV. If transvection is responsible for *trans*-suppression, then neither *γ737* nor *γ648* should be capable of full *trans*-suppression in all tissues, since both are missing enhancers and coding region. Strikingly, both *γ737* and *γ648* are able to suppress *γ878 y*$^+$ variegation to the same level as *γ158*, as quantitated in triple row bristles (98 and 100% relative suppression, respectively), and as observed on the body cuticle. We conclude that *trans*-suppression of *γ878* PEV does not occur via transvection, since derivatives missing regions of the *y* gene required for transvection (GEYER *et al.* 1990) *trans*-suppress as well as a derivative with no major structural change (*γ158*).

Full *trans*-suppression of variegation is disrupted by altered minichromosome structure: Chromosome pairing and homology have been implicated in a number of Drosophila chromosomal phenomena, including PEV (see Introduction). Alterations in chromosome structure can affect chromosome pairing. *Trans*-suppression by minichromosomes with large deletions and inversions were examined to determine if chromosome structure (and potentially chromosome pairing) plays an important role in this phenomenon.

Deletion of a 75-kb region including the y locus disrupts trans-suppression: γ1088 has a 75-kb deletion of the *y*$^+$ gene and the euchromatin surrounding it, while derivative *γ240* has a 185-kb deletion of centric heterochromatin and euchromatin, including the *y*$^+$ gene region (LE *et al.* 1995 and Figure 4A). Both of these chromosomes were able to *trans*-suppress *γ878 y*$^+$ variegation, but only to 50 and 55% relative suppression, respectively. These results indicate that the region surrounding the *y* locus plays an important role in *trans*-suppression.

Very large deletions have a deleterious effect on trans-suppression: Derivatives *γ840* and *γ1230* have very large deletions of both heterochromatin and euchromatin (300 and 700 kb deletions, respectively, see Figure 4A). These minichromosomes cause slight, but significant, *trans*-suppression: 26 and 27% relative suppression, respectively. Thus, minichromosomes with very large deletions (*e.g., γ840* and *γ1230*) *trans*-suppress poorly, in comparison to smaller deletions (*e.g., γ240* and *γ1088*).

Inversions of centric heterochromatin disrupt trans-suppression of γ878 PEV: Chromosomal inversions can influ-

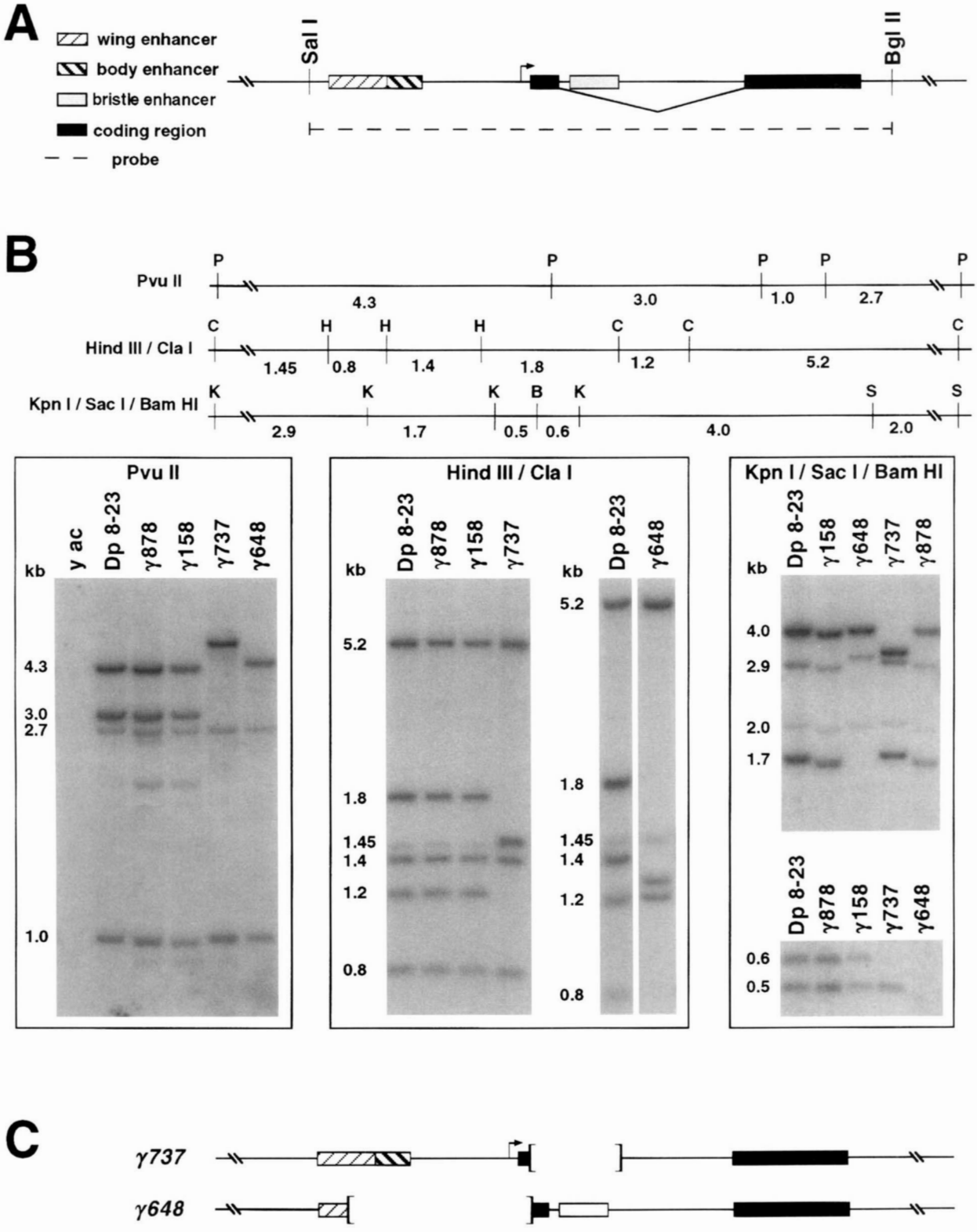

FIGURE 3.—Molecular analysis of the *yellow* locus of y− ry+ *Dp 8-23* derivatives. (A) Diagram of the structure of the *yellow* locus (GEYER and CORCES 1987). (B) Restriction maps and Southern analyses: *Pvu*II; *Hin*dIII + *Cla*I; *Kpn*I + *Sac*I + *Bam*HI. Numbers indicate fragments visualized by the probe (the *Sal*I/*Bgl*II fragment shown in A); fragment sizes are in kilobases (kb). Maps are aligned with the structure of the *yellow* locus in A. In the left panel, lack of hybridization to *y ac* genomic DNA indicates the complete lack of homology between probe and DNA from the *y ac X* chromosome. Extra bands are due to star activity of *Pvu*II. (C) Diagram of the structure of *yellow* in *γ648* and *γ737*.

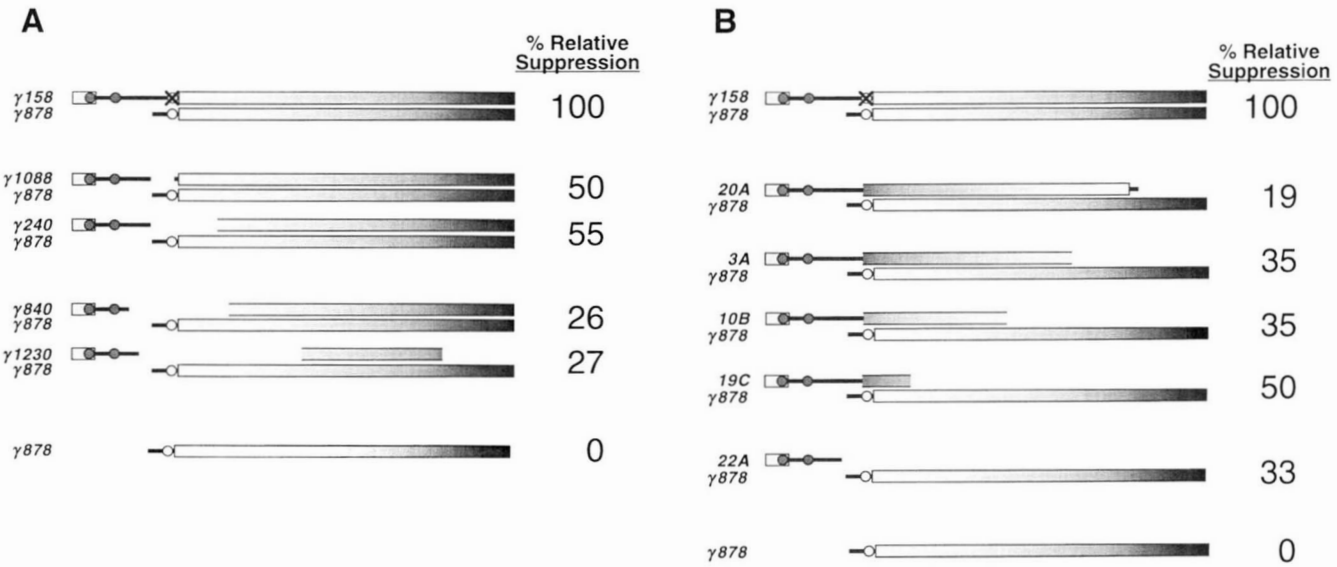

FIGURE 4.—Structures and relative *trans*-suppression of minichromosomes indicating full *trans*-suppression requires chromosome homology: (A) deletions decrease *trans*-suppression. (B) A heterochromatic inversion decreases *trans*-suppression. See text and legends for Figures 1 and 2 for description of derivatives and relative *trans*-suppression.

ence homologue pairing, and thus communication and gene expression (LEWIS 1954; PIRROTTA 1990). We examined the role of centric heterochromatin homology and orientation in *trans*-suppression using a series of *γ238* derivatives. *γ238* is a *Dp 8-23* derivative with a large inversion of centric heterochromatin; one breakpoint is located just distal to *yellow*, and the other within the centric heterochromatin (LE *et al.* 1995; MURPHY and KARPEN 1995).

20A is a derivative of *γ238* that contains 800 kb of centric heterochromatin (Figure 4B) yet *trans*-suppresses *γ878* variegation only slightly, to 19% relative suppression. In contrast, the *Dp 8-23* derivative *γ240* displays 55% relative *trans*-suppression (Figure 4A). *20A* and *γ240* contain similar amounts of *Dp1187* centric heterochromatin; however, the heterochromatic region of *20A* is inverted relative to that of *γ240* and *γ878* (compare Figure 4, A and B). Thus, it appears that the inverted 800 kb of heterochromatin inhibits the ability of *20A* to *trans*-suppress *γ878* PEV.

Support for this hypothesis comes from the study of other *γ238* derivatives that share the same euchromatic breakpoint as *20A*, but have significant deletions of the centric heterochromatin. Derivatives *3A* and *10B* contain 600 and 400 kb of inverted heterochromatin, respectively. Both display ~35% relative suppression, considerably higher than that observed with *20A* (Figure 4B). In addition, *19C*, which contains only 140 kb of centric heterochromatin, *trans*-suppresses to 50%. All these derivatives have the same inversion breakpoint, yet have vastly different levels of *trans*-suppression; thus inhibition of *trans*-suppression is not due to the position of the *γ238* inversion breakpoint near y^+. The surprising observation that derivatives with less inverted heterochromatin are capable of much stronger suppression of *γ878* y^+ PEV indicates that aberrant homology disrupts *trans*-suppression. We conclude that the orientation and amount of centric heterochromatin influences *trans*-suppression.

A derivative with no homology to *γ878* can also *trans*-suppress y^+ PEV: Given the apparent importance of chromosome homology and orientation in *trans*-suppression of *γ878* y^+ variegation, we were interested in seeing whether a minichromosome with no homology to *γ878* would be capable of *trans*-suppression. Derivative *22A* is an acentric terminal deficiency chromosome that is 225 kb in size (Figure 4B). The *22A* terminal breakpoint is ~18 kb distal to the breakpoint of *γ878*; thus the two minichromosomes do not overlap. Nevertheless, *22A* suppresses *γ878* variegation in *trans* (33% relative suppression, Figure 4B). We conclude that although homology is important for *trans*-suppression, other factors also contribute.

DISCUSSION

We have described the *trans*-suppression of position effect variegation associated with a terminal deficiency of the Drosophila minichromosome *Dp1187*. There are a number of advantages to studying PEV in this system. Since these minichromosomes are not required for the viability of the organism, it is possible to induce large changes in the minichromosome and analyze their effect on variegation. In addition, unlike most variegating chromosomes, the overall structure of *Dp1187* and its derivatives have been characterized at the molecular level, due to the relatively small sizes of these minichromosomes (≤1300 kb). Thus, changes in the molecular structure of chromosomes can be correlated directly to long-range effects on gene expression.

Loss of terminal sequences causes enhanced variegation: *In(1)sc*^{8}, *Dp1187,* and *Dp 8-23* all show slight variegation of y^+ (KARPEN and SPRADLING 1990; LINDSLEY and ZIMM 1992) presumably induced by the proximity of a large block of heterochromatin 20 kb proximal to the *y* locus. *γ878* is a terminal deficiency of *Dp 8-23* that has lost 213 kb of distal chromatin, including the telomere, and shows substantial enhancement of variegation for y^+. A number of other terminal deficiencies of *Dp1187* have also been isolated which show a similar enhancement of variegation (TOWER *et al.* 1993; ZHANG and SPRADLING 1993; LE *et al.* 1995). This strongly suggests that loss of terminal sequences is directly responsible for increased *y* variegation, even when the breaks are >100 kb from the *y* locus.

Two observations argue that terminal deficiency-associated PEV is not caused by somatic loss of the minichromosome. First, cytological analyses of a number of minichromosomes with full centromere function (as has *γ878;* MURPHY and KARPEN 1995) indicate no evidence of somatic loss from larval neuroblast tissue (K. COOK and G. KARPEN, unpublished data). Second, if *γ878* PEV were due to somatic loss of the minichromosome, we would expect an extra *Y* chromosome to increase PEV, since the transmission of centromere-defective chromosomes is reduced in the presence of an extra *Y* chromosome (WINES and HENIKOFF 1992; T. MURPHY and G. KARPEN, personal communication). Instead, an extra *Y* chromosome dramatically increases y^+ expression from *γ878.* Therefore, it is unlikely that the variegation phenotype seen at the *γ878* y^+ locus is due to somatic loss of the minichromosome. Later we will address alternative models for terminal deficiency-associated PEV, suggested by our studies of *trans*-suppression.

Although these studies have allowed us to gain an increased understanding of terminal deficiency-associated PEV and of *trans*-suppression, there are a number of issues we cannot address at this time. For example, it is not known in what cells the inactivation of the y^+ gene occurs to create the variegated phenotype. Inactivation and/or reactivation could occur in a determination stage (*e.g.,* early embryos) or in an expression stage (*e.g.,* bristle cells), or both (early developmental analyses reviewed in SPOFFORD 1976; LU *et al.* 1996). In addition, we do not know if terminal deficiency-associated PEV and *trans*-suppression act by altering y^+ gene transcription or gene dosage. Previous data has indicated that the PEV of genes on this minichromosome correlates with the underrepresentation of the locus in polytene tissues (KARPEN and SPRADLING 1990), and it would not be surprising if the y^+ locus of *γ878* were underrepresented in polytene bristle cell nuclei. Indeed, *Dp1187* terminal deficiencies show increased size heterogeneity of chromosome fragments in ovarian tissue, consistent with loss of heterochromatin and the euchromatin adjacent to it (SPRADLING 1993). Regardless of how *y* expression is reduced in *γ878* (*e.g.,* reduced transcription or underrepresentation, or both), the more intriguing issue is why the y^+ locus of *γ878* is more "heterochromatic" than is the y^+ locus of its parent *Dp1187* minichromosome, and why the presence of a second minichromosome in *trans* has such a strong suppressing effect. It is these issues which the experiments described in this article have attempted to address.

Full *trans*-suppression of variegation depends on structural similarity between the two minichromosomes: We have tested and rejected two plausible models for *trans*-suppression. First, *trans*-suppression is not due to the presence of an extra 1000 kb of heterochromatin, since derivatives completely lacking centric heterochromatin (*26C*) suppress well, and some derivatives with large amounts of centric heterochromatin suppress poorly (*20A*). Second, *trans*-suppression is not caused by the type of transvection known to act at the *y* locus (GEYER *et al.* 1990), since derivatives lacking *y* enhancer and coding regions (*γ648* and *γ737*) are capable of *trans*-suppressing to the same high level as a derivative with a point mutation (*γ158*).

Analysis of the suppression mediated by structurally altered *Dp1187* derivatives indicates that overall structural similarity, in both the euchromatic and centric heterochromatic regions, plays an important role in *trans*-suppression. Derivatives with moderately sized deletions (*γ1088* and *γ240*) show a dramatic drop in their ability to *trans*-suppress compared to a full-length minichromosome (*γ158*). Derivatives with very large deletions (and reduced structural similarity) show a correspondingly low ability to *trans*-suppress. A critical observation is that a derivative with inverted centric heterochromatin relative to *γ878* (*e.g., 20A*) shows low *trans*-suppression compared to derivatives with no inversion (*e.g., γ158*). Strikingly, as the amount of inverted heterochromatin is reduced (*3A, 10B* and *19C,* Figure 4B), *trans*-suppression increases. This indicates the orientation of homologous centric heterochromatin is crucial to *trans*-suppression.

We propose that chromosome pairing is an important component of *trans*-suppression. Derivatives with moderate to large deletions may have a lower probability of pairing with *γ878,* which would result in fewer y+ cells. A similar situation may arise with inversion derivatives, which could pair with *γ878* in two different orientations. When inversion derivatives pair in a specific orientation (*i.e.,* where the two euchromatic regions are parallel; see Figure 2 *γ878* and *20A*), *trans*-suppression occurs, and when they pair in the opposite orientation (*i.e.,* where the two heterochromatic regions are parallel), *trans*-suppression does not. Decreasing the amount of centric heterochromatin in the inversion derivatives would increase the probability of pairing that allows *trans*-suppression, and thus increase the amount of *trans*-suppression.

How could chromosome pairing affect y^+ expression in *trans*? We propose two models to explain both termi-

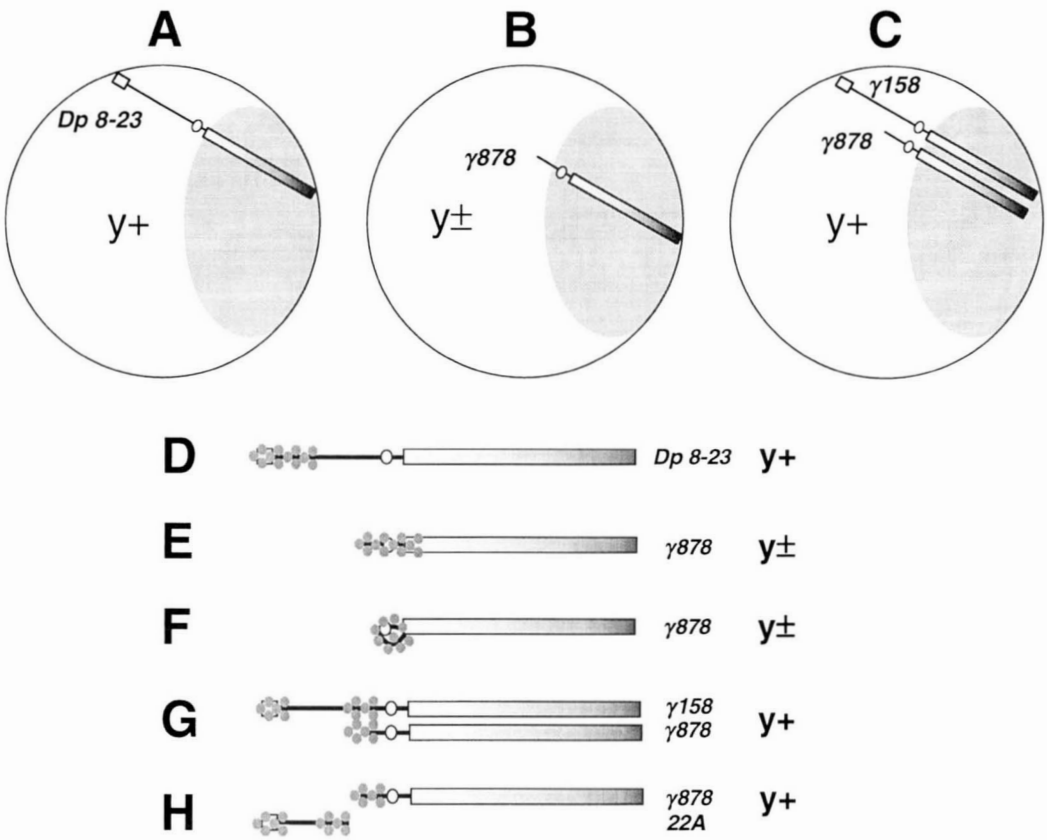

FIGURE 5.—Models for *trans*-suppression. (A–C) Nuclear positioning model. Shaded regions indicate heterochromatic domains; here only a single heterochromatic domain is shown, though in some cell types a single nucleus may contain multiple heterochromatic domains (DERNBURG *et al.* 1996). Unshaded regions indicate euchromatic domains. (A) Full-length minichromosome *Dp 8-23* is positioned within the nucleus such that the y^+ locus is within a euchromatic domain; *y* phenotype is normal (y+). (B) Loss of terminal sequences causes *γ878* to be positioned abnormally, such that the y^+ locus is within a heterochromatic domain; *y* phenotype is variegating (y±). (C) Pairing of *γ878* with full-length *γ158* causes the y^+ locus of *γ878* to be positioned within a euchromatic domain; *y* phenotype is normal (y+). (D—H) Telomere packaging model for *trans*-suppression. (D) Telomere proteins (●) interact with the end of full-length *Dp 8-23*; *y* phenotype is normal (y+). Telomere proteins can also interact with the ends of terminal deficiency *γ878* and either spread to the y^+ locus (E) or cause looping of the tip into the nearby centric heterochromatin (F); in either case, *y* phenotype is variegating (y±). (G) Pairing of *γ878* and *γ158* allows for *trans*-suppression; *y* phenotype is normal (y+). (H) Titration of telomere proteins by minichromosome *22A*; *y* phenotype is normal (y+).

nal-deficiency associated PEV and *trans*-suppression: nuclear positioning and binding of telomeric proteins. Although we discuss the two models separately, they are not completely mutually exclusive.

***Trans*-suppression and a nuclear positioning model:** In certain cell types, chromosomes maintain a specific position within the nucleus, and some data indicate that telomeres appear to play an important role in the positioning and movement of chromosomes (FUNABIKI *et al.* 1993; CHIKASHIGE *et al.* 1994; DAWE *et al.* 1994; DERNBURG *et al.* 1996). Additional studies suggest that the position of a gene within the nucleus can impact its expression (WAKIMOTO and HEARN 1990; KARPEN 1994; HENIKOFF *et al.* 1995; CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996). We propose that loss of terminal sequences may cause *γ878* to be positioned inappropriately within the nucleus. One version of this class of models is shown in Figure 5. Ordinarily, the y^+ locus of full length minichromosomes (such as *Dp 8-23*) is positioned in a euchromatic domain (Figure 5A). Due to loss of terminal regions, *γ878* could undergo frequent, inappropriate associations with a heterochromatic domain that would result in reduced expression of the y^+ gene (Figure 5B). The *γ158* minichromosome, positioned normally within the nucleus, may be able pair with *γ878* and reposition it to a more appropriate location within the nucleus (Figure 5C). Repositioning of *γ878* in individual cells would allow appropriate expression of the y^+ gene, seen as *trans*-suppression. The nuclear positioning model is consistent with our conclusion that only chromosomes capable of faithful pairing with *γ878* induce full *trans*-suppression.

Previous studies have indicated that the PEV of a gene can be affected by the structure of the homologous chromosome and that a homologue can act to pair with and change the position of a chromosome, thereby altering gene expression. HENIKOFF *et al.* (1995) have investigated how alterations in homologue struc-

ture affect *para*-inactivation, the PEV of a bw^+ gene duplication induced by a large piece of heterochromatin in *cis*. A collection of genetic modifiers of *para*-inactivation includes a series of rearrangements of the homologous chromosome. Cytological analyses of the rearranged chromosomes indicated that in every case where the rearranged homologue enhanced *para*-inactivation, the bw^+ locus of the homologue was moved closer to heterochromatin. HENIKOFF *et al.* (1995) posit that pairing occurs between the homologues and allows the subsequent "dragging" of the bw^D chromosome closer to a heterochromatic domain. Although the details differ between our study of *Dp1187 trans*-suppression and that of HENIKOFF *et al.* (1995), there are sufficient similarities to suggest the two phenomena may be working via a similar mechanism. The association of the bw^D locus with heterochromatin has been shown cytologically (HENIKOFF *et al.* 1995; CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996). Future cytological studies will determine whether a similar mechanism is responsible for *trans*-suppression of *γ878*.

Our nuclear positioning model implies that *trans*-suppression occurs through chromosome pairing. How then does derivative *22A*, which has no obvious region of overlap with *γ878*, act to *trans*-suppress? It is possible that *22A* and *γ878* share common features that promote chromosome pairing and hence *trans*-suppression, such as repeated sequences or proteins that bind different DNA sequences, yet interact. In either case, *22A* could position *γ878* appropriately within the nucleus, thus inducing *trans*-suppression. Alternatively, *22A* may be acting via a separate, but potentially overlapping mechanism, as described below.

***Trans*-suppression and binding of telomeric proteins:** A second model to explain *Dp1187* terminal deficiency-associated PEV postulates that proximity to telomeric proteins interferes with the normal expression of the $\gamma 878\, y^+$ locus. Telomeres are required for a number of functions, including protecting chromosome ends from endonuclease degradation and preventing fusion of chromosome ends (MASON and BIESSMANN 1995; ZAKIAN 1995). Drosophila termini do not contain the short, simple telomeric repeats found in most eukaryotes. Rather, Drosophila telomeres are composed of transposable elements (*e.g.*, HeT-A and TART) (LEVIS *et al.* 1993; WALTER *et al.* 1995), and other types of repeated DNA (*e.g.*, TAS repeats) (KARPEN and SPRADLING 1992; THOMPSON-STEWART *et al.* 1994; WALTER *et al.* 1995). It is believed that such arrays are produced in part by rare terminal transposition of the transposable elements onto the ends of chromosomes, since HeT-A and TART elements are also found at the tips of some terminal deficiency chromosomes (BIESSMANN *et al.* 1990b; SHEEN and LEVIS 1994). We extend the suggestion of BIESSMANN and MASON (1988) and propose that all ends of Drosophila chromosomes, including those of recovered terminal deficiencies, are packaged as telomeres. Recoverable terminal deficiencies in Drosophila contain at least some telomere functions: they are not extensively degraded, nor do they undergo measurable frequencies of chromosome fusion, even though in most cases their termini do not contain transposable elements (BIESSMANN and MASON 1988; LEVIS 1989; BIESSMANN *et al.* 1990a). In addition, terminal deficiencies in Drosophila can be transmitted normally from parent to offspring (MURPHY and KARPEN 1995), thus they do not display the instability expected of dicentrics created by the fusion of unprotected terminal deficiency chromosomes (MCCLINTOCK 1938). The packaging of terminal deficiency ends as telomeres could protect the chromosomes from end fusion and endonuclease digestion.

Packaging may also make any terminus heterochromatic with respect to gene expression. PEV is frequently seen in transgenes located just proximal to telomeres of yeast (GOTTSCHLING *et al.* 1990; NIMMO *et al.* 1994) or in subtelomeric regions in Drosophila (LEVIS *et al.* 1985; KARPEN and SPRADLING 1992; TOWER *et al.* 1993; WALLRATH and ELGIN 1995; ROSEMAN *et al.* 1995). Telomeric silencing in *S. cerevisiae* is thought to occur via "spreading" of silenced chromatin (RENAULD *et al.* 1993; PALLADINO and GASSER 1994; HECHT *et al.* 1995). A similar mechanism may be occurring on *γ878*. Telomeric proteins present at the new terminus may spread to the *y* locus, thus reducing its expression (compare Figure 5, D and E). Alternatively, gene inactivation could occur through the "looping" of the new telomeric heterochromatin to interact with the nearby heterochromatin 20 kb proximal to the y^+ gene, similar to the proposed mechanism for bw^D variegation (TALBERT *et al.* 1994; CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996) (Figure 5F). In either case (spreading or looping), we propose that increased proximity of the chromosome end to the y^+ locus induces variegation and any interference with the action of the tip on the y^+ gene causes suppression of variegation. Pairing of *γ878* with a second minichromosome may inhibit the activity of telomeric proteins, perhaps through spreading of telomeric proteins onto the homologous chromosome (Figure 5G). The terminus-proximity model is consistent with reduced *trans*-suppression by chromosomes less capable of pairing with *γ878* and is especially consistent with the large impact of deletions in the vicinity of *y*.

Our data indicate that altered chromosome homology disrupts *trans*-suppression, and suggest that somatic pairing is an important component. However, we also have evidence that *trans*-suppression occurs in the absence of any chromosome homology, for example, in the presence of acentric chromosome *22A*. One attraction of the terminus packaging model is that it can account for this seemingly contradictory observation. The addition of two more termini (one telomere, one terminal deficiency end) included by the presence of

22A may titrate away limited telomere proteins and therefore reduce the level of variegation (Figure 5H). A similar mechanism has been described in yeast; the addition of extra telomeric sequences on a linear plasmid can act in *trans* to suppress the position effect of a gene located in the vicinity of a chromosomal telomere (WILEY and ZAKIAN 1995).

Increased variegation is not a general property of all terminal deficiencies. A series of terminal deficiencies of the *X* chromosome have been recovered, none of which show variegation for *y* (MASON *et al.* 1984; BIESSMANN and MASON 1988). Terminal deficiencies have also been demonstrated to suppress the PEV of marker genes inserted into subtelomeric regions (LEVIS 1989; TOWER *et al.* 1993; SHEEN and LEVIS 1994); in all reported cases of suppression, the terminal break has been just outside the *P* element, implying that sequences immediately distal to the *P* element (the conserved repeats that flank all of these insertions) are directly responsible for these special cases of PEV. It may be that the extreme variegation seen in *Dp1187* terminal deficiencies is due to the combined effects of both the terminal deficiency and the centric heterochromatin just proximal to the y^+ locus, a situation present on neither the normal *X* nor the *P* insertion chromosomes described above. Proximity to centric heterochromatin may cause the minichromosome y^+ locus to be more sensitive to other PEV-inducing factors. It is also possible that the very end of a chromosome may be packaged differently than the subterminal region. Proteins at the very tip may not cause silencing; perhaps only the subterminal domain is packaged by proteins that cause gene silencing.

We have identified a novel chromosomal phenomenon, the *trans*-suppression of terminal deficiency-associated PEV. Our molecular-genetic analyses suggest that full *trans*-suppression requires chromosomal pairing, but a limited level of suppression can occur without pairing. We have proposed two models to explain *trans*-suppression: nuclear positioning and activity of telomere proteins. These two mechanisms are not necessarily mutually exclusive nor are they necessarily additive; it is possible that for any particular minichromosome *trans*-suppression may occur via one or both mechanisms. Future studies will focus on testing the models for *trans*-suppression via cytological, molecular, and genetic approaches. For example, the nuclear positioning model makes specific predictions about the location of the y^+ gene in different variegating and *trans*-suppressed genotypes, which can be investigated with recent advances in cytological technologies (CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996). In addition, proteins responsible for terminal deficiency-associated variegation and *trans*-suppression can be identified using genetic modifier screening. By determining the normal roles of such proteins in nuclear function, we can test the importance of both the somatic pairing and telomere packaging models. Investigation of this unique and manipulable system should contribute to our understanding of the importance of nuclear positioning and chromosome pairing, the metabolism of chromosome ends, and the long-range regulation of gene expression.

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